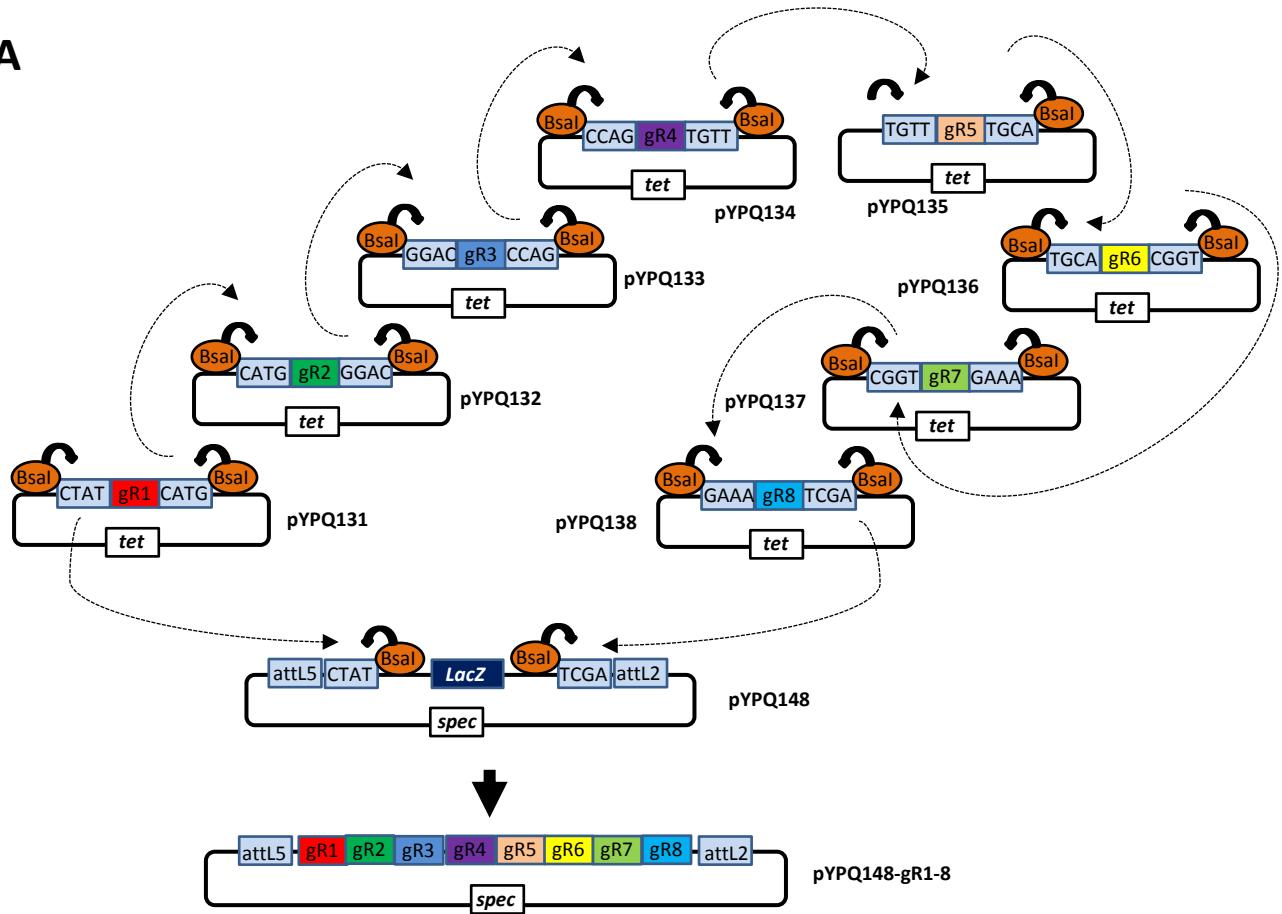
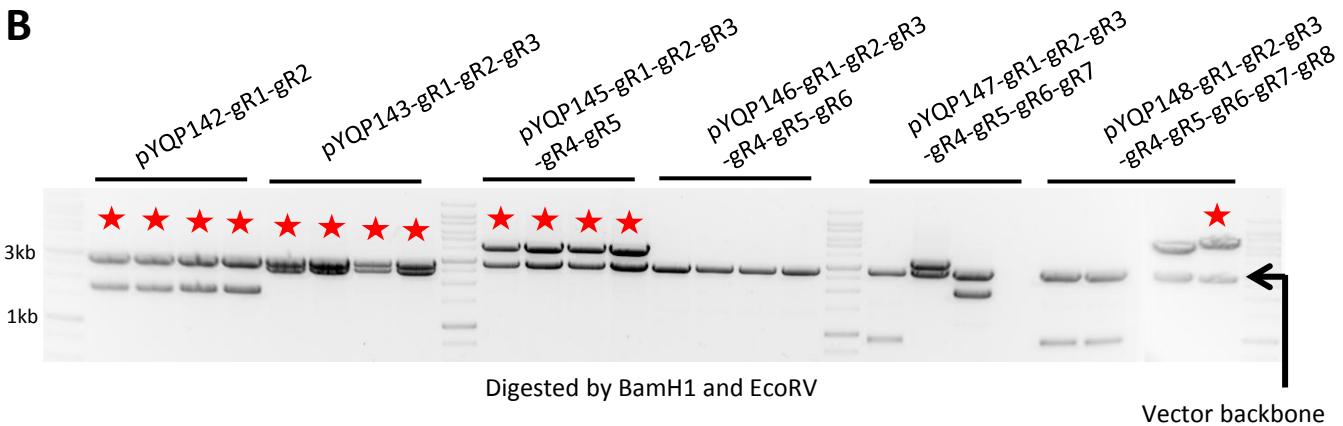
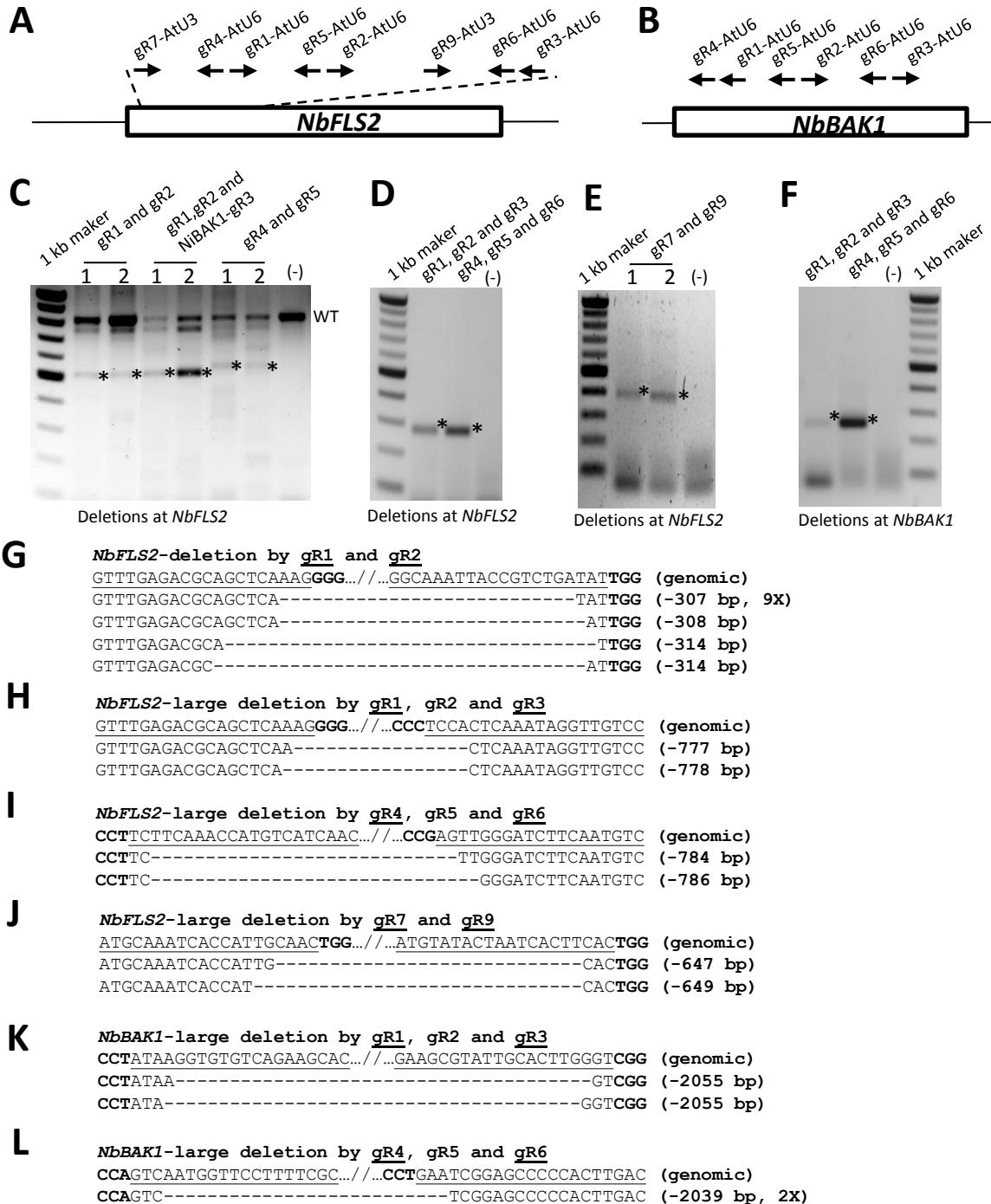


**Figure S1. Architecture of Cas9 proteins in the toolbox**

Architecture of different Cas9 proteins is depicted. Note these Cas9 proteins differ from each other based on different epitope tag or effector protein fusions.

**A****B****Figure S2. Golden Gate assembly of up to 8 gRNA expression cassettes**

A, A depiction of Golden Gate assembly of 8 gRNA expression cassettes into one recipient vector based on compatible ends resulting from *BsaI* digestion. B, Restriction digestion of plasmids derived from randomly selected white colonies (on blue/white selection LB plate) to confirm Golden Gate assembled vectors. Note the successfully assembled vectors that contain 2, 3, 5 and 8 gRNA expression cassettes are indicated by red stars.



**Figure S3. Targeted chromosomal deletions at *NbFLS2* and *NbBAK1* in *N. benthamiana***

Schematics in (A) and (B) depict target genes and relative positions of gRNA binding sites in *NbFLS2* and *NbBAK1* respectively. C-E, PCR based detection of deletions at *NbFLS2*. The deletion bands are indicated by asterisks. Note the larger PCR products are corresponding to wild type (WT) sequence. The numbers indicate biological replicates and the wild type control plant is labeled as (-). F, PCR based detection of deletion at *NbBAK1*, with the deletion bands indicated by asterisks. G-L, Sequence confirmation of different lengths of deletions at *NbFLS2* and *NbBAK1* induced by different gRNA pairs.